Calcasieu Estuary Remedial Investigation/Feasibility Study (RI/FS): Baseline Ecological Risk Assessment (BERA)

Appendix F2: Assessment of Risks to Carnivorous Fish in the Calcasieu Estuary

Prepared For:

CDM Federal Programs Corporation
8140 Walnut Hill Lane, Suite 1000
Dallas, Texas 75231

Under Contract To:

Mr. John Meyer, Regional Project Manager
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Prepared – October 2002 – By:

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Under Contract To:

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CONTRACT NO. 68-W5-0022
DOCUMENT CONTROL NO. 3282-941-RTZ-RISKZ-14858

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Appendix F2. Assessment of Risks to Carnivorous Fish in the Calcasieu Estuary

1.0 Introduction

Development and industrialization in and around the Calcasieu Estuary in southwestern Louisiana in recent decades has led to concerns of environmental contamination in the area. A Remedial Investigation/Feasibility Study (RI/FS) was commissioned to determine the risks posed by environmental contamination to ecological receptors inhabiting key areas of the Calcasieu Estuary. A Baseline Ecological Risk Assessment (BERA) is required to meet this objective. This Appendix is a part of the BERA and is conducted in accordance with the procedures laid out by the USEPA in the *Ecological Risk Assessment Guidance for Superfund: Process for Designing and Conducting Ecological Risk Assessment* (USEPA 1997). Under the eight-step process described by the USEPA for conducting a BERA, a screening ecological risk assessment (SERA) must first be conducted to determine preliminary estimates of exposure and risk.

The SERA for the Calcasieu Estuary (CDM 1999) identified areas of concern, chemicals of potential concern (COPCs), and ecological receptors potentially at risk. The SERA findings were revisited in a Baseline Problem Formulation (BPF) to yield a refined list of chemicals of potential concern (COPCs), areas of interest, and ecological receptors to be considered in the BERA. The Phase II data collection provided more information and, therefore, a better tool to estimate risk at a screening level. Using this information a conservative, deterministic assessment was conducted and can be found in Appendix G along with a description of the methods used to identify the contaminants of concern (COCs) and areas of concern for carnivorous fish.

This Appendix is organized as follows. Section 1 provides an overview of the results of the conservative, deterministic ERA for carnivorous fish including the Areas of Concern (AOCs) and COCs that screened through (see Appendix G for details). Section 1 also includes a description of the conceptual model for carnivorous fish. Section 2 briefly describes the methods and data used in the tissue residue analysis from Phase II. Section 3 describes the probabilistic risk assessment methods used to estimate risks of COCs to carnivorous fish in the Calcasieu AOCs. Section 4 describes the probabilistic risk assessment results and conclusions. Section 5 identifies sources of uncertainty that could influence the estimated risks for carnivorous fish.

1.1 Deterministic Ecological Risk Assessment Summary

The methods and results of the deterministic ecological risk assessment are presented in detail in Appendix G. In summary, the deterministic risk assessment used a conservative approach to estimate risk to carnivorous fish from COPCs in the Bayou d'Inde, Upper, and Middle regions of the Calcasieu Estuary system. Several reference sites, including Bayou Connine Bois and Choupique Bayou, were also included in the deterministic assessment to provide a basis for comparison of risk. The deterministic assessment compared potentially attainable high exposures with conservative adverse effects benchmarks to provide a means of identifying which contaminants were a concern to carnivorous fish and in which areas of the Calcasieu Estuary system. A risk quotient (tissue residue level / tissue residue effect benchmark) for carnivorous fish greater than one for any COPC in any of the

Calcasieu areas resulted in the COPC, subsequently called a contaminant of concern, being screened through to the probabilistic ecological risk assessment.

Total polychlorinated biphenyls (total PCBs) were screened in for Bayou d'Inde AOC, Upper Calcasieu River AOC and Middle Calcasieu River AOC. The reference areas were also included in the probabilistic ecological risk assessment so that risks in the AOCs could be compared to background risks. Results of the deterministic risk assessment are presented in Table F2-1.

1.2 Areas of Concern

Based on the results of the SERA (CDM 1999), the portion of the Calcasieu Estuary from the saltwater barrier to Moss Lake was identified as the area in which environmental contamination posed the greatest potential risks to ecological receptors and, as such, was designated as the primary study area. To facilitate the RI/FS, this study area was divided into four sub-areas (termed Areas of Concern, AOCs) and two reference areas, including:

- Upper Calcasieu River AOC (UCR AOC);
- Bayou d'Inde AOC (BI AOC);
- Middle Calcasieu River AOC (MCR AOC);
- Sabine National Wildlife Refuge; and
- Other Reference Areas

Each of the areas of concern were further divided into subareas. Bayou d'Inde AOC includes Upper Bayou d'Inde, and Middle Bayou d'Inde. The Upper Calcasieu River AOC includes Coon Island Northeast, Coon Island Southwest, Clooney Island Loop, and Lake Charles. The Middle Calcasieu River AOC includes Prien Lake, Citigo Surge Pond, Old River Channel, Indian Marias Lagoon, and Moss Lake. There are several sub-areas that make up the Reference Areas. These include Bayou Connine Bois, Choupique Bayou, Grand Bayou and Wetlands, Johnsons's Bayou, Calcasieu Lake, and Sabine National Wildlife Refuge. Bayou Verdine was not included in this risk assessment because risk management strategies have already been agreed upon by the USEPA and affected parties for this area of concern.

The conservative, deterministic screening ecological risk assessment for wildlife and carnivorous fish (Appendix G) determined that the AOCs were limited to Bayou d'Inde AOC, Middle Calcasieu River AOC, and Upper Calcasieu River AOC. Comparisons of tissue residue levels to effects benchmarks were done for AOCs rather than sub-areas to ensure adequate sample size.

1.3 Chemicals of Potential Concern

For carnivorous fish total PCBs were determined to have a hazard quotient above one in several AOCs. A description of this COC is provided below.

Total Polychlorinated Biphenyls (total PCBs)

Polychlorinated Biphenyls (PCBs) are anthropogenic synthetic organic chemicals that are persistent, bioaccumulative, and biomagnify in the food chain. PCBs are widespread contaminants and when released to aquatic systems tend to partition into and become incorporated into sediments (MacDonald *et al.* 2001). They are created when chlorine atoms replace hydrogen atoms on a biphenyl structure. The biphenyl structure is composed of two benzene rings, joined by a single carbon-carbon bond. There are ten positions where chlorine atoms can join the biphenyl structure. As a result, there are 209 variations, or PCB congeners, that can be created. Only about 130 of the 209 congeners occur in synthesized mixtures of PCBs (USEPA 2001; McFarland and Clarke 1989). PCBs are hydrophobic compounds with log K_{ow}s ranging from 4.40 to 8.18 (Niimi 1996). Dissolved concentrations of PCBs in water near their solubility levels are unlikely to occur, particularly due to their hydrophobic behavior and affinity to absorb to suspended particulates, sediment and biota (Niimi 1996). Aqueous solubility ranges from 1 mg/L to the low :g/L range generally depending upon the level of chlorination of the congener. Niimi (1996) in a review of the literature reported that PCB concentrations measured in aquatic organisms can vary by a factor of 100000, and the largest differences in PCB concentrations in aquatic biota are found in carnivorous fish when comparing sites with low contamination to those with high contamination.

In the environment, PCBs are usually found as specific mixtures of congeners. Mixtures made by the Monsanto Company are known by the trade name Aroclor. Other commercial trade names of PCB mixtures manufactured outside of the United States include Kanechlor, Clophen, Fenclor, and Phenclor (ATSDR 1997). The congener make-up of each Aroclor determines the physical-chemical properties of the mixture. Aroclors are defined by a four digit number. The first two digits are usually 12. The last two digits represent the percentage by weight of chlorine in the mixture (Environment Canada 1997). For example, Aroclor 1254 contains 54% chlorine by weight. Aroclor 1016 is an exception to this rule. It is a re-distilled version of Aroclor 1242 and contains 41% chlorine (Safe 1994). Aroclor 1254 and Aroclor 1260 were regularly detected in most environmental media and biota in the Calcasieu Estuary AOCs.

Historically, total PCBs have been reported as the sum of the Aroclor values (Newman *et al.* 1998; Sather *et al.* 2001) where Aroclor determination is based on comparison to an Aroclor standard. This was due to an inability to discern individual congeners. The analytical determination of Aroclors does not take into account physiological (metabolization of congeners) nor spatial or temporal changes (i.e., environmental weathering) in the Aroclor mixtures. These processes can modify mixture toxicity (Newman *et al.* 1998; Sather *et al.* 2001). The preferred analytical method for determination of total PCBs is to sum the PCB congeners (Boon *et al.* 1997; McFarland and Clarke 1989; Newman *et al.* 1998; Sather *et al.* 2001). This method accounts for the weathering and metabolic processes that can modify toxicity and is particularly relevant to concentrations in higher trophic level organisms (Boon *et al.* 1997; Sather *et al.* 2001). Congener-specific methods avoid the need to determine which Aroclor most closely fits the detected congener profile in biota or media samples, and does not require assumptions to be made about congener metabolism and weathering. Roughly 20% of the media and biota samples from the Calcasieu Estuary sampling programs (i.e., Phase I and Phase II) were analysed for PCB congeners. In whole body fish and aquatic invertebrates, only 23 PCB congeners were reported. Although not all 209 PCB congeners are required for total PCB determination, McFarland and Clarke (1989) did recommend, however, the inclusion of 36 PCB congeners considered the most relevant to environmental samples. The second preferred method of total PCB determination is to sum the PCB homologs detected in the sample. Each homolog contains PCB congeners with the same number of chlorine atoms (USEPA 2001). By summing the homologs, the metabolic and weathering processes are taken into account and no determination is required regarding the appropriate Aroclor profile. To determine the PCB homologs, congener analysis is required. As only a small subset of congeners (~23) was reported in the Calcasieu samples, this method could not be used to determine total PCB concentrations.

A review of the analytical results from the biota samples collected in Phase II of the Calcasieu sampling program, revealed that Aroclor 1016, Aroclor 1221, Aroclor 1232, and Aroclor 1242 were not detected in any aquatic invertebrate and whole body fish samples collected from the AOCs. Because non-detect values can contribute significantly to the total PCB concentration, if half of the detection limit is used for non-detects, these Aroclors were excluded from the total PCB calculation. Aroclor 1254 and Aroclor 1260 were detected in tissue samples in all AOCs and the Reference Areas. Aroclor 1254 contains 54% chlorine compared to 60% chlorine in Aroclor 1260. These two Aroclors have very similar congener compositions (Frame *et al.* 1996) and as such the analytical resolution of mixtures containing these two Aroclors overlaps (i.e., the same congeners are found in both Aroclors). To account for this overlap, results for each Aroclor were compared on a sample-by-sample basis, and the Aroclor with the highest result or method detection limit was used to estimate total PCB concentration in the sample.

1.4 Receptors of Concern

Carnivorous fish are an integral part of the fish community in freshwater, estuarine, and marine ecosystems. They may also be important prey species for piscivorus (fish-eating) wildlife, including reptiles, birds, and mammals. There are a variety of freshwater fish species that use habitats in the Calcasieu Estuary, particularly in the headwater areas of the various bayous. During the winter and spring, when high rainfall and runoff produce low salinity conditions, freshwater fish species have a wider distribution, in some cases use habitats as far south as Calcasieu Lake. Some of the freshwater species commonly observed within the watershed include spotted gar (*Lepisosteus oculatus*), gizzard shad (*Dorosoma cepedianum*), pugnose minnow (*Notropis emiliae*), blacktail shiner (*Notropis venustus*), blue catfish (*Ictalurus* *furcatus*), channel catfish (*Ictalurus punctatus*), mosquitofish (*Gambusia affinis*), bluegill (*Lepomis macrochirus*), longear sunfish (*Lepomis megalotis*), and white crappie (*Pomoxis annularis*; Felley 1987a; 1987b). Largemouth bass (*Micropterus salmoides*) and chain pickerel (*Esox americanus*) have also been observed within the freshwater portion of the estuary (Felley 1987b).

Estuarine fish species use habitats in Calcasieu Lake and throughout the estuarine portions of the bayous (Felley 1987a). The commonly observed species that fall within this category include ladyfish (*Elops saurus*), gulf menhaden (*Brevoortia patronus*), sheepshead minnow (*Cyprinodon variegatus*), gulf killifish (*Fundulus grandis*), sailfin molly (*Poecolia latipinna*), inland silverside (*Menidia beryllina*), chain pipefish (*Syngnathus louisianae*), hogchoaker (*Trinectes maculatus*), bay whiff (*Citharichthys spilopterus*), and naked goby (*Gobiosoma bosci;* Felley 1987b).

A variety of marine fish species occur in the Calcasieu Estuary during a portion of their life history. Some of the species commonly encountered in the estuary include black drum (*Pogonias cromis*), red drum (*Sciaenops ocellatus*), pinfish (*Lagodon rhomboides*), sheepshead (*Archosargus probatocephalus*), sand seatrout (*Cynoscion arenarius*), spotted seatrout (*Cynoscion nebulosus*), silver seatrout (*Cynoscion nothus*), Atlantic croaker (*Micropogonias undulatus*), spot (*Leiostomus xanthurus*), striped mullet (*Mugil cephalus*), white mullet (*Mugil curema*), hardhead catfish (*Arius felis*), gafftopsail catfish (*Bagre marinus*), bay anchovy (*Anchoa mitchilli*), and southern flounder (*Paralichthys lethostigma*; Felley 1987a; 1987b). Even such species as tarpon (*Megalops atlanticus*), cobia (*Rachycentron canadum*), Atlantic stingray (*Dasyatis americana*), southern kingfish (*Menticirrhus americanus*), and Atlantic spadefish (*Chaetodipterus faber*) have been periodically observed in the estuary (Felley 1987b).

Four focal species were identified in the BERA and are the focus of this Appendix. They include: spotted seatrout, black drum, red drum, and southern flounder (MacDonald *et al.* 2001). The life histories and foraging behavior of each of these species are summarized below.

Spotted Seatrout (*Cynoscion nebulosus*)

Spotted seatrout are carnivores that are found in shallow coastal marine waters, often over sand bottoms (Robins and Ray 1986). They frequent inland bays in the summer and move to deeper bays in the fall, when the water temperature declines (Texas System of Natural Laboratories 1991a). These migrations are short term and many studies have found this species to remain within a five mile radius of the spawning site (Texas System of Natural Laboratories 1991a).

From March through October, female seatrout spawn between six and eight times (Holt and Holt 2000; Tucker and Faulkner 1987). Spawning occurs in the deeper waters of bays and estuaries where the water is less turbulent and small larvae are often found in seagrass beds (Holt and Holt 2000). The larvae are demersal and range from 1.3 to 1.56 mm in length (Fable *et al.* 1978). Nieland *et al.* (2002) captured male (n=601) and female (n=1451) spotted seatrout from Barataria Bay, Louisiana. Seatrout age ranged from 0.48 to 5.14 years based on otoliths annulus counts. The body weight (mean ± SD) for the males ranged from 70 - 1050 g (mean=330 ± 185.6 g) and ranged from 80 - 2090 g (mean=558 ± 283.3 g) for females.

Seatrout have a diurnal feeding pattern (Holt and Holt 2000) and, as adults, feed on shrimp and fish, especially mullet (Hoese and Moore 1998). Large seatrout often eat fish of near equal size and as a result seatrout eat only once or twice a week (Hoese and Moore 1998). Holt and Holt (2000) found that calanoid copepods, bivalve larvae, copepod egg sacs and gastropod viligers were the most important prey items in large, seatrout larvae.

Black Drum (*Pogonias cromis*)

Black drum are benthic carnivores and one of the largest sciaenids. They are long lived and weigh up to 68 kg (Nieland and Wilson 1993). They are demersal and are found over sand or soft bottoms, often in areas with large river runoffs (Scotton *et al.* 1973). Black drum are predominantly a bay species, with young commonly inhabiting shallow, nutrient rich and muddy bodies of water (Texas System of Natural Laboratories 1991b). Black drum can tolerate a wide range of temperatures and salinities, but are most commonly found in hypersaline conditions (Simmons and Breuer 1962).

When the water temperature reaches approximately 26 degrees Celsius, between January and early April, black drum spawn in bays and in or near connecting passes (Simmons and Breuer 1962; Robins and Ray 1986). After spawning, they quickly return to their preferred bay habitat (Simmons and Breuer 1962). Females have a spawning lifetime ranging from 15 to 30 years, with annual fecundities of 13 to 67 million ova (Nieland and Wilson 1993). Black drum reach sexual maturity at the end of the second year, when they are approximately 320 mm long (Simmons and Breuer 1962). Length data collected from the Calcasieu Estuary Phase II sampling program were used to calculate fish weights for the samples collected. Seventeen black drum whole body fish samples were found in the Calcasieu v21 database (CDM 2002). Body weights ranged from 106 - 1680 g. Three additional black drum weights were located in Simmons and Breuer (1962) for three age 1-3 year fish. Body weights ranged from 54 - 982 g. The mean of these fish (n=21) was 429 ± 372 g.

The diet of adult black drum consists of shrimp, molluscs and some fish, taken from the bottom (Scotton *et al.* 1973; Simmons and Breuer 1962). The young consume mainly marine annelids, small fishes and soft crustaceans (Simmons and Breuer 1962).

Red Drum (*Sciaenops ocellatus*)

Red drum are a carnivorous species found in large, offshore schools (Hoese and Moore 1998). However, estuaries and inshore oceanic waters are inhabited by red drum for feeding and for the first three to four years of their life (Mercer 1984).

From August through October, red drum migrate to deeper waters to spawn near the mouths of bays and inlets (Holt and Holt 2000). Through tidal streams, their eggs and larvae are transported to estuaries, where they settle into seagrass beds (Holt and Holt 2000). The young remain there from six months to three or four years of age (Simmons and Breuer 1962). Growth occurs rapidly in the early stages of life and adult red drum reach a maximum length of 155 cm (Reagan 1985). Length data collected from the Calcasieu Estuary Phase II sampling program were used to calculate fish weights for the samples collected. Thirty-one whole body red drum samples were selected from the Calcasieu database v21 (CDM 2002). Body weights ranged from 464 - 6277 g. Additional whole body weights were located from several sources (i.e., Simmons and Breuer 1962; Murphy and Taylor 1990; Froese and Pauly 2002). These were added to the thirty-one fish located in the Calcasieu database and the mean body weight for red drum was calculated as 2240 ± 2435 g (n=79). In this dataset red drum were a minimum of 1 year old. The complete age range is unknown as not all of the fish were aged.

Adult red drum have a diurnal feeding pattern (Holt and Holt 2000) and primarily consume fish, shrimp and crab (Mercer 1984). They feed from shallow waters, over sandy to muddy bottoms (Mercer 1984). The diet of the juvenile red drum consists of small bottom invertebrates and young of other fish (Mercer 1984). Holt and Holt (2000) found that dinoflagellates and calanoid copepods, with widths from 10 to 280 :m, were the most important prey in large, red drum larvae.

Southern Flounder (*Paralichthys lethostigma*)

Southern flounder are carnivores found in estuaries and coastal waters, mostly over mud bottoms, at depths to approximately 40 m (Frimodt 1995). They can be found in coastal waters ranging from fresh to salt water (van Maaren *et al.* 2001) and have been found at salinities between 0 and 36 ppt (Daniels 2000).

Flounder migrate offshore in the fall, to spawn during December and January (van Maaren *et al.* 2001), and return to estuaries immediately following spawning (Daniels 2000). Females spawn approximately 100,000 eggs per kg of body weight, over several days (Daniels 2000). The larvae remain offshore for between 30 to 60 days until metamorphosis begins (Daniels 2000). After metamorphosis and the migration of the right eye to the left side of the head, flounder begin to migrate into estuaries and up rivers (Daniels 2000). Female flounder grow approximately three times faster than male flounder (Daniels 2000). At two years of age, when the flounder reach sexual maturity, females weigh from 800 to 1000 g and males from 300 to 400 g (Daniels 2000).

Adult flounder feed aggressively at the surface and consume anchovies, menhaden, sciaenids and mullet (Reagan 1985). The juveniles feed primarily on invertebrates, making up 95 percent of their diet, among which mysids are consumed 32 percent of the time (Reagan 1985).

1.5 Conceptual Model

The conceptual model illustrates the relationships between COC fate and behavior, sources and releases, and the exposure pathways through which carnivorous fish are exposed. The model enhances the level of understanding regarding the relationships between human activities and ecological receptors at the site under consideration. In so doing, the conceptual model provides a framework for predicting effects on ecological receptors and a template for generating risk questions and testable hypotheses (USEPA 1997; 1998b). The Baseline Problem Formulation (BPF) contains a complete site conceptual model for the Calcasieu Estuary. It summarizes information on the sources and releases of COCs, the fate and transport of these substances, the pathways by which ecological receptors are exposed to COCs, and the potential effects of these substances on ecological receptors that occur in the Calcasieu Estuary. In turn, this information is used to develop a series of risk hypotheses that provide predictions regarding how ecological receptors will be exposed to and respond to COCs.

Based on the pathways identified in the site conceptual model, diet is likely the most important route of exposure for carnivorous fish for bioaccumulative substances such as total PCBs (Oliver and Niimi 1988; Holm *et al.* 1993; MacDonald *et al.* 2001; USEPA 1998a). Fish are potentially at risk from very low concentrations of highly bioaccumulative substances such as PCBs in surface water due to the large volume of water (10 -100 L/day) processed across the oxygen exchange surface of the gills (Oliver and Niimi 1988). Very low concentrations of these substance in surface water can therefore cause significant accumulation in fish tissues. An analysis of surface water concentrations of total PCBs in the Calcasieu Estuary indicated that surface water concentrations were not above the method detection limit in any of the areas of concern. This is not unexpected as total PCBs partition quickly to sediment

(MacDonald *et al.* 2001a). The primary exposure of fish to PCBs in sediment comes from foraging, nest building or resting, and through incidental ingestion. Relative to dietary contributions, the contribution of sediments to PCB residues in carnivorous fish is considered minor (Oliver and Niimi 1988). Therefore, surface water and sediment exposure were not specifically evaluated for their potential for adverse effects on carnivorous fish in the estuary. Surface water and sediment pore water exposure were considered for non-carnivorous fish in the fish communities assessment (Appendix F1).

1.6 Assessment Endpoints

In this Appendix, we estimate risks of total PCBs on the survival, growth, and reproduction of carnivorous fish.

1.7 Measurement Endpoints

Data on levels of total PCBs in carnivorous fish will be compared to effects concentrations to determine the probability of adverse effects on survival, growth, or reproduction.

1.8 Risk Hypothesis and Questions

The following risk hypothesis was developed to identify the key stressor-effect relationships that will be evaluated in the probabilistic ecological risk assessment:

> Based on the physical-chemical properties of the bioaccumulative substances of concern, the nature of the food web in the Calcasieu Estuary, and the effects that have been documented in laboratory and field studies, PCBs released into surface waters will accumulate in the tissues of aquatic organisms to levels that adversely affect the survival, growth, and/or reproduction of carnivorous fish.

To provide a basis for assessing ecological effects, the assessment endpoint must be linked to the measurement endpoint by risk questions. In this assessment, the investigation to assess the effects of environmental contaminants on carnivorous fish were designed to answer the following risk questions:

- Are the levels of PCBs in the tissues of carnivorous fish in the Calcasieu Estuary higher than tissue residue benchmark values for survival, growth and reproduction?
- If yes, what are the probabilities of effects of differing magnitude for survival, reproduction, and/or growth of carnivorous fish?

1.9 Purpose of Assessment

The purpose of this assessment is to test the above risk hypothesis and answer the risk questions by characterizing risks posed to the carnivorous fish community associated with exposure to the COCs identified in Appendix G.

2.0 Methods

A step-wise approach was used to assess risks to carnivorous fish posed by the COCs in the Calcasieu Estuary. The five main steps in this process included:

- Identification of assessment endpoints, risk questions and testable hypotheses, and measurement endpoints (Sections 1.6 to 1.8);
- Collection, evaluation, and compilation of the relevant information on the concentrations of COPCs in carnivorous fish in the Calcasieu Estuary (Section 2);
- Selection of benchmarks and effects metrics for COPCs (Appendix G and Section 3.2);
- Implementation of a deterministic assessment of risks to carnivorous fish, including the identification of chemicals of potential concern (COPCs) and areas of concern (see Appendix G); and,
- Implementation of a probabilistic assessment of risks to carnivorous fish for those COCs and AOCs that screened through the deterministic assessment (Section 3).

Each of these steps is described in this report and flow charts outlining each step are provided in Figures F2-1 to F2-3. The results of the deterministic assessment were briefly reviewed in Section 1.1. For details of this assessment, see Appendix G.

2.1 Collection, Evaluation, and Compilation of Data

Information on chemical levels in tissues of carnivorous fish were collected in two phases, termed the Phase I and Phase II sampling programs. The Phase I program results indicated that the detection limits for many of the COPCs in tissues were orders of magnitude above corresponding benchmarks. Therefore, the Phase I results for tissues were not considered in this assessment. The methods used to collect the Phase II tissue samples, quantify the levels of COPCs, evaluate the reliability of the data, and compile the information in a form that would support the BERA are described in the following sections.

Sample Collection of Tissues **-** More than 600 tissue samples were collected at sites located throughout the estuary between October, 2001 and November, 2001. Biota tissue samples were collected in three AOCs in the estuary (UCR AOC, MCR AOC, and BD AOC) and in the reference areas (Bayou Connine Bois, Calcasieu Lake, Choupique Bayou, Grand Bayou and Grand Bayou and Wetlands, Sabine National Wildlife Refuge). There were also a number of sub-areas within the AOCs from which samples were taken. The USEPA Region V FIELDS tools were used to randomly select coordinates (i.e., latitude and longitude) for the assigned number of primary sampling stations and alternate sampling stations (i.e., which were sampled when it was not possible to obtain samples from the primary sampling stations). In the field, each sampling station was located with the aid of navigation charts and a Trimble differentially-corrected global positioning system (GPS). Using standard statistical power analysis methods, an evaluation of previously collected data was completed to determine the number of samples to be collected within each area and sub-area.

The methods used to collect, handle, and transport the tissue samples are described in CDM (2000a; 2000b; 2000c; 2000d; and 2000e). Briefly, fish species were collected by hook and line, hand collection and netting. Minnows and other small bait species were collected using legal cast nets, minnow traps, dip nets and bait seines in accordance with the Louisiana Department of Wildlife and Fisheries. Each sample was wrapped in aluminum and put in a Ziploc® bag. All samples were kept frozen and shipped to laboratories in coolers on dry ice.

Chemical Analyses of Tissues - Chemical analysis of the tissue samples was conducted at various contract laboratory program (CLP) and subcontract (non-CLP) analytical laboratories, including USEPA Region VI Laboratory, USEPA Region VI CLP laboratories, Olin Contract laboratories, Texas A&M University laboratories, ALTA laboratories, AATS laboratories and EnChem laboratories. Upon receipt at the laboratory, tissue samples were held in freezers until analysis.

All tissue samples were analyzed for total target analyte list (TAL) metals, target compound list (TCL) semi-volatile organic compounds (SVOCs) and TCL pesticides. Total metals were quantified using the SW6010B method. Polycyclic aromatic hydrocarbons and/or other semi-volatile organic compounds were quantified using the SW8270C method. Methods SW8081A and SW8082 were used to quantify pesticides. Twenty percent of the tissue samples were analyzed for PCB congeners and dioxins and furans. EPA Method SW1668 was used to quantify PCB congeners and SW8290 was used for dioxins and furans.

EnChem laboratories used additional analytical methods to quantify mercury, polycyclic aromatic hydrocarbons (PAHs), pesticides and dioxins and furans. Methods 1631MOD and 1630MOD were used to quantify mercury and methylmercury, respectively. PAHs were quantified using Method 8270C-SIM. Method SW8082 and AXYS Method CL-T-1668A/Ver.3 were used to quantify pesticides. Dioxins and furans were quantified using AXYS Method DX-T-8290/Ver.2.

Data Validation and Verification - All of the data sets generated during the course of the study were critically reviewed to determine their applicability to the assessment of risks to the biotic community in the Calcasieu Estuary. The first step in this process involved validation of the tissue chemistry data. Following translation of these data into database format, the validated data were then further evaluated to ensure the quality of the data used in the risk assessment. We were unable to confirm tissue data results against the original source.

Database Development - To support the compilation and subsequent analysis of the information on biota in the Calcasieu Estuary, a relational project database was developed in MS Access format. All of the sediment chemistry data compiled in the database were georeferenced to facilitate mapping and spatial analysis using geographic information system (GIS)-based applications (i.e., ESRI's ArcView and Spatial Analyst programs). The database structure made it possible to retrieve data in several ways, including by data type (e.g., chemistry vs. toxicity), by stream reach (e.g., UBI vs. LBI), by sub-reach (e.g., UBI-1 vs. UBI-2), and by date (e.g., Phase I vs. Phase II). As such, the database facilitated a variety of data analyses. No database was created for the tissue data, rather, the CDM (2002) database was used to access the tissue data available.

3.0 Probabilistic Ecological Risk Assessment

3.1 Exposure Characterization

The number of whole body fish tissue samples available for each carnivorous fish species is reported in Table F2-2 along with length ranges for the fish. A total of 96 carnivorous fish samples were analyzed for total PCBs from the AOCs. Variability in total PCB fish tissue concentrations may be a result of several factors including lipid content and foraging patterns of the individual fish. Lipid content is likely the largest individual source of variability because PCBs partition to lipids and lipid content is highly variable between fish of differing sizes and sex. By normalizing tissue concentrations to lipid content, this source of variability can be reduced. Age of the fish can still be a factor after lipid normalization as older fish have had more opportunity to accumulate PCBs over time. The diet preferences of the fish is also a large source of variability between species. Each of the four focal species have similar diet preferences, however, the availability and preference of certain prey vary both spatially (i.e., prey from specific areas) and temporally (i.e., preferences for higher trophic level prey at different times of the year) and can add to variability among species. Summary statistics using both wet weight (ww) and lipid normalized (LN) tissue residues will be used to describe the general trends in fish body burdens found in the each of the AOCs.

3.1.1 Observed Tissue Concentrations

Geometric mean tissue concentrations of total PCBs in carnivorous fish collected from the areas of concern (i.e., Bayou d'Inde AOC, Middle Calcasieu River AOC, Upper Calcasieu River AOC) are summarized in Table F2-3. Very few data were available for southern flounder in all AOCs. The two flounder sampled from the Reference Areas had a mean concentration of 0.11 mg/kg (ww).

Whole body fish tissue concentrations (ww) of total PCBs in Bayou d'Inde AOC ranged from non-detect levels (MDL=0.005 mg/kg) to 0.530 mg/kg. The highest mean concentrations were found in spotted seatrout in this AOC (Table F2-3). Only one value below the method detect limit (MDL) (0.005 mg/kg) was reported for black drum. On a mean lipid normalized basis, PCB concentrations in the red drum and spotted seatrout had the highest concentrations (6.97 ± 12.4 mg/kg lipid and 6.07± 23.8 mg/kg lipid, respectively). High total PCB concentrations in the black drum would be anticipated as they are long lived fish and have the opportunity to accumulate higher residues over time. Unfortunately, only one black drum was sampled in Bayou d'Inde AOC, where PCB sources are known to exist (CDM 1999)

In the Upper Calcasieu River AOC, fish tissue total PCB concentrations (whole body) ranged from 0.01 to 1.1 mg/kg (ww) in all fish species. On a wet weight basis, black drum had the highest tissue residue concentration in this AOC. On a lipid normalized basis, spotted seatrout had the highest mean total PCB residue (9.02 mg/kg lipid) of the three fish species captured.

Mean MCR AOC total PCB fish tissue residues ranged from 0.008 to 0.129 mg/kg ww. The highest tissue residues on a wet weight (0.129 mg/kg) and lipid normalized (25.98 mg/kg) basis were found in the black drum.

Fish sampled from the Reference Areas had tissue residues ranging from 0.007 to 0.035 mg/kg (ww). The highest mean concentrations were detected in spotted seatrout. On a lipid normalized basis, the means ranged from 4.12 to 23.6 mg/kg lipid for all fish, with spotted seatrout having the highest mean concentration (i.e., 23.6 mg/kg lipid).

3.1.2 Spatial and Temporal Differences Between AOCs

On a wet weight basis, mean fish tissue residues of total PCBs do not markedly differ between the AOCs (Table F2-3). The mean total PCB concentration (mean ± SD) for fish in Bayou d'Inde AOC (n=34) was 0.0369 ± 0.0953 mg/kg (ww). On a wet weight basis this was the highest mean concentration of the AOCs. The Upper Calcasieu River AOC fish samples had a mean total PCB tissue residue concentration of 0.0339 ± 0.219 mg/kg (ww) (n=25). This is only slightly lower than Bayou d'Inde AOC. Middle Calcasieu River AOC had a mean tissue concentration of 0.030 ± 0.172 mg/kg (ww) (n=17), while the Reference Areas had a mean concentration of 0.0129 ± 0.0205 mg/kg (ww). There was a marked difference between the AOCs in terms of lipid normalized mean total PCB concentration in fish tissue. The Reference Areas had a mean total PCB tissue concentration of 10.2 ± 18.0 mg/kg lipid. Upper Calcasieu River AOC had a mean concentration of 6.75 ± 19.2 mg/kg lipid. Bayou d'Inde AOC and Middle Calcasieu River AOC had mean concentrations of 5.97 ± 19.2 mg/kg lipid and 5.91 ± 175 mg/kg lipid, respectively. The extremely high standard deviation for Middle Calcasieu is due to a fish sample with a low lipid content and a relatively high PCB concentration. On a lipid normalized basis, the Reference Areas had the highest mean total PCB tissue residue concentrations (10.2 ± 18.0 mg/kg lipid). The range of lipid content in fish in each of the AOCs is as follows: Bayou d'Inde AOC 0.1 - 4.5 %; Upper Calcasieu River AOC 0.1 - 2.5%; Middle Calcasieu River AOC 0.1 - 3%; and Reference Areas 0.1 - 0.5%. The range of lipid content of the fish in the Reference Areas indicates that the fish sampled did not have very high lipid contents relative to the fish in the other AOCs. This had the effect of increasing the total PCB content calculated in these fish on a lipid normalized basis.

Historical data

Levels of Aroclor 1254 in tissues of fish collected from CH2M Hill's Calcasieu Estuary Biological Monitoring Program were consistent with levels found in the Phase II Sampling Program. Levels in whole body determined in 2001 during Phase II Sampling and levels in fillet recorded since 1991 by CH2M Hill were used for statistical analysis. For comparison, fillet concentrations were estimated for the samples collected from the Phase II Sampling Program using the following equation:

$$C_f = C_{wb} / 2.3 \qquad \textbf{Equation 1}$$

where, C_{wb} is whole-body concentration and C_f is fillet concentration (SAIC 1993).

Annual geometric mean concentrations in fillet of red drum, black drum, spotted seatrout, sand seatrout and southern flounder were calculated for the four AOCs. The geometric mean concentration of Aroclor 1254 in fillet collected from the Upper Calcasieu River AOC during the Phase II Sampling Program was 0.013 mg/kg, with minimum and maximum concentrations of 0.002 mg/kg and 0.478 mg/kg, respectively. Since 1991, the annual geometric mean concentrations determined by CH2M Hill's Biological Monitoring Program ranged from 0.006 mg/kg to 0.040 mg/kg and the minimum and maximum concentrations were 0.005 mg/kg and 0.232 mg/kg, respectively (Figure F2- 4).

The geometric mean concentration of Aroclor 1254 in fillet collected from the Bayou d'Inde AOC during the Phase II Sampling Program was 0.016 mg/kg, with minimum and maximum concentrations of 0.002 mg/kg and 0.230 mg/kg, respectively. Since 1991, the annual geometric mean concentrations determined by CH2M Hill's Biological Monitoring Program ranged from 0.028 mg/kg to 0.133 mg/kg and the minimum and maximum concentrations were 0.003 mg/kg and 1.080 mg/kg, respectively (Figure F2-5).

The geometric mean concentration of Aroclor 1254 in fillet collected from the Middle Calcasieu River AOC during the Phase II Sampling Program was 0.013 mg/kg, with a minimum and maximum concentration of 0.002 mg/kg and 0.317 mg/kg, respectively. Since 1991, the annual geometric mean concentrations determined by CH2M Hill's Biological Monitoring Program ranged from 0.008 mg/kg to 0.031 mg/kg and the minimum and maximum concentrations were 0.003 mg/kg and 0.221 mg/kg, respectively (Figure F2-6).

The geometric mean concentration of Aroclor 1254 in fillet collected from the Calcasieu Estuary reference areas during the Phase II Sampling Program was 0.006 mg/kg, with a minimum and maximum concentration of 0.002 mg/kg and 0.029 mg/kg, respectively. Since 1991, the annual geometric mean concentrations determined by CH2M Hill's Biological Monitoring Program ranged from 0.006 mg/kg to 0.016 mg/kg and the minimum and maximum concentrations were 0.003 mg/kg and 0.378 mg/kg, respectively (Figure F2-7).

The comparison of historical data sets between the Phase II Sampling Program and CH2M Hill's Biological Monitoring Program showed that there was less than one order of magnitude difference in levels of total PCBs in fish tissue between the ten years of historical data and data collected in the Phase II Sampling Program. In most cases, the difference was less than four fold. This demonstrates that the results of the ecological risk assessment for carnivorous fish using data from the Phase II Sampling Program are likely to be temporally representative.

3.1.3 Estimates of Exposure

Whole body burdens of total PCBs in carnivorous fish are likely a result of dietary uptake (Oliver and Niimi 1988). Estimates of total daily intake were not calculated for carnivorous fish because of the lack of acceptable toxicity studies using dietary exposures for total PCBs (see Section 3.2). For this assessment, levels of total PCBs reported in the tissues of the fish species were used as a measure of whole body burden of the COPC regardless of route of uptake. There are several advantages to this approach. The use of whole body burdens is considered a more direct measure of the potential for adverse effects (Sijm *et al.* 1993; van Wezel *et al.* 1995; Jarvinen and Ankley 1999). The use of fish whole body tissue residues also removes much of the uncertainty associated with total daily intake (TDI) calculation. For example, there is no need to estimate proportion and species of prey in the diet, metabolic rate in the fish, gross energy of the prey, time spent feeding in the Estuary and other factors (e.g., spatial and temporal factors) that add to uncertainty in the TDI model. There are disadvantages to using this approach however. First, a sufficient number of fish must be sampled to provide some confidence that the exposure statistics generated for each COC are representative of the levels in the fish population found in the AOC. Secondly, if the level of the COC in the AOC is sufficiently elevated the measured body burden distribution for sampled fish may be truncated as fish with higher body burdens will have been removed from the sample population (i.e., they are dead). This is of concern at sites with extremely high COC concentrations.

3.2 Effects Characterization

The purpose of this section is to: (1) briefly review the literature on the effects of total PCBs to carnivorous fish, and (2) select the appropriate effects metric that will be used with the results of the exposure assessment to estimate risk. Our focus will be on the assessment endpoints of growth, reproduction and survival of carnivorous fish.

3.2.1 Measures of Effect

Effects data can be characterized and summarized in a variety of ways ranging from benchmarks designed to be protective of most or all species to dose-response curves for the receptor group of interest (e.g., carnivorous fish). Jarvinen and Ankley (1999) developed a comprehensive database of studies that examined the link between tissue residue values and effects in fish from exposure to inorganic and organic chemicals. The conservative, deterministic risk assessment (Appendix G) used tissue residue toxicity reference values (TRV) for fish taken from Jarvinen and Ankley (1999). The TRV chronic value (TRV_{ChV}) was the geometric mean of the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL). When a fish TRV for a COPC could not be estimated using the Jarvinen and Ankley (1999) database, one was calculated using the USEPA freshwater chronic criterion or acute marine criterion and multiplying this by a measured or estimated BCF to produce a fish TRV_{NOAEL}. This was not necessary for total PCBs as there were several appropriate studies available. The study by Berlin *et al.* (1981) was used to generate a TRV_{ChV} of 0.43 mg/kg total PCBs for use in the conservative, deterministic risk assessment (Appendix G).

A literature search was undertaken to identify studies that could add to the body of information available in Jarvinen and Ankley (1999) and to assist in further characterizing the potential effects of total PCBs to carnivorous fish. The literature search was not limited to only those studies that reported tissue residue levels and subsequent effects in fish. Rather, any study available that examined the impact of total PCBs (as Aroclor 1254/1260 or total PCBs) to fish was considered. Several excellent reviews of the scientific literature for effects of PCBs to fish species were found (e.g., USEPA 1998a; Monosson 1999; Meador *et al.* 2002; USEPA 2000) and used in this assessment. Each of the studies identified in the literature search were reviewed for appropriateness, data quality and completeness. This process included determining if appropriate controls were used (and control mortality was acceptable), appropriate statistics were applied, standard laboratory protocols were used, and measured concentrations were reported. Only those studies that reported whole body tissue residue data were included to facilitate comparison to available exposure data. The literature search also focused on studies that had reproduction, growth and survival as endpoints and used an appropriate analyte (i.e., total PCBs, 3PCB congeners, Aroclor 1254 and Aroclor 1260). Field studies were discussed in the text as supporting evidence for effects reported in laboratory studies. Field studies were not used to derive benchmarks due to the presence of other chemicals in the field. Similarly, studies that examined the impact of egg tissue PCB residues were excluded for use in deriving benchmarks. Residue data collected in the Calcasieu Estuary did not include fish eggs, and comparison to adult whole body burdens is uncertain. To compare egg residues to adult body burdens, at a minimum, the data must be compared on a lipid normalized basis. There is uncertainty related to the lipid content of the eggs if not measured in laboratory studies. This uncertainty arises because lipid content in eggs varies between species, season, reproductive phase of the fish, and is influenced by food availability. There is uncertainty regarding which form of lipid (i.e., polar or non-polar) bioaccumulative substances preferentially accumulate into (Meador *et al.* 2002). It is unclear whether polar and non-polar lipids in eggs are present in the same ratio as those in adults. Additionally, Mac *et al.* (1993) reported that there were differences in congener patterns between adult fish and their eggs, suggesting preferential deposition of certain congeners in the eggs. Therefore, Ah-receptor mediated effects and non Ah-receptor mediated effects cannot be considered equally between the two tissue types when comparing to only adult whole body burden data from the Calcasieu Estuary. Finally, studies are reviewed that report reproductive effects based on adult or juvenile body burdens. Thus, this endpoint is already considered in the assessment.

Injection studies are not considered to be environmentally relevant in this assessment. Monosson (1999) reviewed the literature to compare injection study body burdens in fish with body burdens accumulated through contaminated media, diet, and maternal transfer. Injection studies were found to result in a more precise body burden in the fish based upon the injection dose (i.e., between 50 - 70% of the injected dose was measured in the tissue). Dietary and media exposure generally resulted in ~50% of the dose level subsequently being measured in the tissue (Monosson 1999). The difference between the two could be explained by taking into account assimilation efficiency and possibly metabolic changes to the PCB as it passed through the gastrointestinal tract of the fish. An injection of PCBs is essentially an acute exposure, thus effects can occur prior to redistribution of the PCBs to other tissues. For these reasons, injection studies were not considered in this Appendix other than as ancillary information.

There were no toxicity studies found in the literature that were specific to any of the focal species in this Appendix. Where appropriate, results from studies for other fish species (both marine and freshwater) will be discussed. A summary of the effects of total PCBs to fish is provided below. Studies deemed acceptable for use in the development of the effects metric are summarized in Table F2-4 and Figure F2-8.

Overview

Adverse effects to fish reported in field and laboratory studies include reduced survival, growth, and reproduction, as well as abnormal behavioral responses and biochemical alterations (reviewed in Niimi 1996; Monosson 1999; Eisler 2000). Several dioxin-like PCB congeners have the same toxic mechanism as 2,3,7,8-TCDD. This toxicity mechanism involves the binding of these co-planar congeners to the aryl hydrocarbon (Ah) receptor and elicitation of an Ah receptor-mediated biochemical and toxic response (van den Berg *et al.* 1998; Newsted *et al.* 1995; Safe 1994). In fish, the 2,3,7,8-TCDD and equivalent congeners are generally associated with early life stage mortality (Wisk and Cooper 1990; Walker *et al.* 1994). Non-Ah receptor-mediated biochemical and toxic responses are also known to occur in fish from exposure to total PCBs (reviewed in Monosson 1999). These effects are typically related to altered hormone and neurotransmitter levels and may also be characterized as endocrine effects. The endocrine system is an important regulating and integrating system that relies on hormone production, transport and action to control growth, development and reproduction functions (Monosson 1999).

Survival

The most sensitive test result for survival was reported by Berlin *et al.* (1981) for lake trout (*Salvelinus namaycush*) exposed to Aroclor 1254. This study was the basis for the carnivorous fish benchmark developed in the conservative, deterministic risk assessment for wildlife and carnivorous fish (Appendix G). Berlin *et al.* (1981) exposed lake trout fry hatched from eggs collected from Lake Michigan to PCB concentrations in water and diet. After 176 days of exposure, PCB body burdens were 1.53 mg/kg, 5.06 mg/kg, and 26.3 mg/kg (ww) in the 10, 50, 250 ng/L treatments respectively. Each of the fish receiving a water exposure also received total PCB doses in food containing 1, 5 and 25 mg/kg during the 176 day study. After 96 days, mortality was significantly different (35.4% mortality) from controls in the lowest exposure treatment (corresponding to a body burden of 1.53 mg/kg). Hansen *et al.* (1973) exposed pinfish (*Lagodon rhomboides*) juveniles to concentrations of Aroclor 1254 in water for 14 days. Whole-body tissue residues of 14 mg/kg were found to cause a 66% decrease in survival of the juvenile fish. Mauck *et al.* (1978) measured brook trout (*Salvelinus fontinalis*) survival after 127 days exposure to Aroclor 1254 in water. No effect was observed to hatching time, egg hatchability, or sac-fry mortality. Survival was compromised at tissue residue levels of 125 mg/kg (21% reduction in survival). Exposure kinetics can play a significant part in the toxicity/tissue residue relationship in fish. For example, van Wetzel *et al.* (1995) examined the lethal body burdens of Aroclor 1242 to fathead minnows (*Pimephales promelas*) from exposure to contaminated water. After one day (water concentration = 89.6 to 138.9 :g/L) mortality was observed and whole body fish tissue residues ranged from 1.28 to 20 mg/kg in the dead fish. Fish that survived the initial exposure but perished six days later had tissue residue concentrations of 102 to 256 mg/kg.

Reproduction

Reproductive effects observed in laboratory studies included delayed spawning, reduced fecundity, and reduced hatchability. Freeman and Idler (1975) exposed brook trout to a water concentration of 0.2 mg/L Aroclor 1254 for 21 days. After 21 days, back muscle tissue contained 32.8 mg/kg PCB, while eggs were found to contain 77.9 mg/kg. The hatching success of eggs was reduced 22% relative to controls. This study demonstrates the importance of maternal transfer of PCBs to eggs. Only the adults (male and female) treated with Aroclor 1254 subsequently had the reduced hatching success relative to the control fish. Unfortunately, an experimental error prevented the determination of whether the reduced hatchability was due to PCB effects on sperm or eggs directly (Freeman and Idler 1975). Similar results were observed in a study by Mac *et al.* (1993) where embryonic mortality was found to be related to PCB residues in the eggs and adult tissue of lake trout. Many authors believe that reabsorption of the yolk sac at swim-up in many fish species may be the most sensitive of life stages (Wisk and Cooper 1990; Walker *et al.* 1994). Maternal transfer of PCBs may also have its greatest impact at this life stage as bioaccumulative contaminants will likely be found in high concentrations in the lipids of the yolk sac.

Hansen *et al.* (1973) examined the impact of Aroclor 1254 on sheepshead minnow by exposing adult minnows to various concentrations in water. Fish were exposed for 28 days in a flow-through bioassay prior to egg induction, and eggs were fertilized and placed in PCB-free flowing seawater and observed for mortality. The sheepshead minnow body burden of 1.9 mg/kg and 9.3 mg/kg were found to be the NOAEL and LOAEL for fry survival. These adult tissue body burdens corresponded to concentrations of 0.88 and 5.1 mg/kg in the eggs. Fry survival to one week of age was 77% for eggs from adults from the 0.32 : g/L treatment (average 9.3 mg/kg in tissue of females), as compared to 95% survival of fry from control adults and 97% survival of fry from adults from the NOAEL treatment (0.1 : g/L; average 1.9 mg/kg in tissue of females).

Mac *et al.* (1993) examined the impact of PCBs on survival in lake trout eggs. In this field study, lake trout were collected from the Great Lakes and females stripped of eggs. The eggs were fertilized with milt from male trout. The eggs were then transferred to clean laboratory water and observed through to hatching. The authors found that there was a relationship between PCB concentration in eggs and embryonic mortality. Measured egg concentrations ranged from 0.25 to 7.77 mg/kg ww while adult whole body burdens ranged from 4 to 14 mg/kg ww. Similar effects were reported in several other field studies with comparable egg tissue concentrations (Giesy *et al.* 1986; Ankley *et al.* 1991; Mac and Schwartz 1992).

Growth

Effects on fish growth have been examined by a number of authors (Mayer *et al.* 1977; Nestel and Budd 1975; Lieb *et al.* 1974; Mauck *et al.* 1978; Mac and Seelye 1981; Nebeker *et al.* 1995). Growth effects reported included reduced bone development, reduced body weights, and reduced fork lengths of several fish species. Mac and Seelye (1981) reported no effect on growth to lake trout fry exposed to 50 ng/L Aroclor 1254 in water and a dietary exposure of 0.72 mg/kg. The tissue residues in the fish ranged from 2-4 mg/kg. Fathead minnows exposed to relatively high Aroclor 1254 concentrations of 4.6 :g/L in water were not affected at tissue residue values ranging from 741 - 1253 mg/kg (Nebeker *et al.* 1995). Hendricks *et al.* 1981 exposed adult rainbow trout to Aroclor 1254 in the diet for two months prior to spawning. Fish were fed the diet containing 200 ppm (mg/kg) Aroclor 1254 twice daily. Eggs were then stripped from the females and incubated to hatching. Measured tissue Aroclor 1254 residues were 0.47 mg/kg in the eggs from control fish and 1.64 mg/kg in the females fed the PCB diet. Adult whole body burdens were 11.4 mg/kg lipid and 45.3 mg/kg lipid in controls and females exposed to Aroclor 1254, respectively. Adult whole body burdens on a lipid normalized basis were converted to a whole body weight basis using an average lipid percentage from Meador *et al.* (2002) of 8.82% for rainbow trout (geometric mean of 5 laboratory based studies). Therefore, PCB concentrations were estimated to be 1.29 and 5.14 mg/kg ww. Growth (measured as body weight relative to control fish) was measured for up to 12 months post hatch. The authors observed a significant decrease in the growth of the embryos from the adult females exposed to Aroclor 1254 relative to the control (28% reduction after 9 months and 20% reduction at 12 months).

Black *et al.* (1988) examined adult winter flounder (*Pseudopleuronectes americanus*) collected from contaminated estuarine environments (e.g., Buzzards Bay, MA and Upper Narragansett Bay, RI) to determine impacts on flounder progeny. Eggs from Buzzards Bay flounder were found to have PCB concentrations of 40 mg/kg dw (~8 mg/kg ww). Larvae hatched from the contaminated eggs in a clean laboratory setting were found to be smaller in length and weight than fish hatched from eggs with lower concentrations from other sites. Linear regression analysis indicated that there was an inverse relationship between PCB concentration in the eggs and length and weight at hatch (Black *et al.* 1988).

3.2.2 Effects Metrics

PCB toxicity occurs over a large range of concentrations with lethal and sublethal effects sharing the same range (Figure F2-8). The most sensitive study was that of Berlin *et al.* (1981) using lake trout fry. A lowest adverse effect level of 1.53 mg/kg in tissue was found to impact survival of lake trout fry. Mac and Seelye (1981) using lake trout fry reported no effect on survival or growth in fry at a tissue residue of between 2 and 4 mg/kg. There were no other studies that reported effects at such low tissue residue levels for any fish species and life stage for which data were available (Figure F2-8; Table F2-4). The Berlin *et al.* (1981) study was therefore considered to be an outlier and is not considered further in this assessment.

For this assessment, a threshold range was developed based on the lowest reasonable no effects value for sensitive species and the highest no effects value for tolerant species. The lowest no effect value selected was from the study by Mac and Seelye (1981) using lake trout fry. Both survival and growth were examined and no effect was observed in the range of 2-4 mg/kg in the tissue of the fry. The 4 mg/kg value was used as the low tissue residue value (TRV_L). The highest no effect value selected was from the study by Nestel and Budd (1975) in which rainbow trout were exposed to PCBs in the diet for 330 days. The study reported a no effects concentration of 81 mg/kg in tissue for survival and growth and is considered to be the high tissue residue value (TRV_H). Slight changes in behavior and pigmentation were observed in the fish at this tissue concentration. This threshold range encompasses a wide range of no effects and effects data from a number of early life stage studies (Hansen *et al.* 1971; Freeman and Idler 1975; Mayer *et al.* 1977; Mauck *et al.* 1978; ACOE 1988). Maternal transfer which may be an important factor in early life stage effects, is considered in several studies (e.g., Freeman and Idler 1975; ACOE 1988).

4.0 Risk Characterization

In the risk characterization phase of the probabilistic risk assessment, the results of the exposure assessment (i.e., reverse cumulative distribution functions) and effects assessment (i.e., threshold range) were integrated to estimate the probabilities of exceeding the lower and upper bounds of the threshold range for black drum, red drum, spotted seatrout, and southern flounder in each AOC. Ideally, a risk characterization includes three major lines of evidence: comparison of exposure to lab-derived effects metrics, *in situ* or whole-media toxicity tests, and biological surveys. For carnivorous fish, the latter two lines of evidence were not available. We therefore rely on the comparison of measured tissue levels to laboratory-derived effects metrics.

4.1 Results

The tissue residue data for black drum, red drum, spotted seatrout, and southern flounder were combined for each AOC. Lognormal distributions were fit to the combined data for each AOC using the maximum likelihood estimation method. We used these distributions to estimate probabilities of exceeding lower and upper bounds of the threshold range. The mean (±SD) body burden was 0.079 ± 0.14 mg/kg. Figure F2-9 depicts the probability of the TRV_L (4 mg/kg) and TRV_H (81 mg/kg) being exceeded for fish in Bayou d'Inde AOC. The probability of the lower TRV being exceeded is 0.01%. The probability of the higher TRV being exceeded is essentially zero. In the Reference Areas, the mean body burden was 0.022 ± 0.020 mg/kg. Figure F2-10 depicts the probability of the TRV_L and TRV_H being exceeded by fish in the Reference Areas. The probability of the lower TRV and higher TRV being exceeded is zero. In the Upper Calcasieu River AOC, the mean total PCB body burden was 0.083 ± 0.16 mg/kg. The probability of the lower TRV being exceeded in the Upper Calcasieu River AOC is 0.02% (Figure F2-11). The probability of exceeding the higher TRV is essentially zero. In the MCR AOC, the mean body burden was 0.095 ± 0.27 mg/kg. The probability of the lower TRV being exceeded was 0.06% (Figure F2-12). There is a 0% probability of the TRV_H being exceeded.

4.2 Conclusion

Based on the results of the risk characterization, there is little likelihood of either the low or high bounds of the threshold range (TRV_L or TRV_H) for total PCBs being exceeded in any of the areas of concern for carnivorous fish. Therefore, the risk of total PCBs to carnivorous fish in the areas of concern is considered low.

5.0 Uncertainty Analysis and Data Gaps

There are a number of sources of uncertainty in the assessment of risk to carnivorous fish, including uncertainty in the conceptual model, and in the exposure, effects, and risk assessments. As each of these sources of uncertainty can influence the risk estimates, it is important to describe and, when possible, quantify the magnitude and direction of such uncertainties. This provides a measure of the level of confidence in the assessment. The uncertainties associated with the assessment of risk to carnivorous fish are described in the following sections.

Uncertainties Associated with the Conceptual Model - The conceptual model is intended to define the links between stressors, potential exposure, and predicted effects on ecological receptors. As such, the conceptual model provides the scientific basis for selecting assessment and measurement endpoints to support the risk assessment process. Potential uncertainties arise from the lack of knowledge regarding ecosystem functions, failure to adequately address spatial and temporal variability in the evaluations of sources, fate, effects, omission of stressors, and overlooking secondary effects (USEPA 1998b). The types of uncertainties associated with the conceptual model that links contaminated sources to effects on carnivorous fish include those associated with identifying ecological effects, and receptors at risk. The identification of appropriate ecological effects likely represents the primary source of uncertainty in the conceptual model. To minimize the effect of selecting one very sensitive effect threshold to compare exposure to, lower and upper bound thresholds were used. The receptors chosen as representative of carnivorous fish in the Calcasieu Estuary are likely to be the dominant high trophic level fish found in the estuary.

Uncertainties Associated with the Exposure Assessment - The exposure assessment is intended to describe the actual or potential co-occurrence of stressors with receptors. It identifies the exposure pathways and the intensity and extent of contact with stressors for each receptor or group of receptors at risk. There are a number of potential sources of uncertainty in the exposure assessment including analytical errors, extrapolation errors, and data gaps.

Chemical analysis of tissue residues in whole body carnivorous fish were used to evaluate exposure of fish to total PCBs. Analytical errors and descriptive errors represent potential sources of uncertainty. Three approaches were used to address these uncertainty sources.

First, analytical errors were evaluated using information on the accuracy, precision, and detection limits (DL) generated to support the Phase II sampling programs. The results of this analysis indicated that the data used in this assessment met the project data quality objectives (see Appendix B1 for more details). Second, all data entry, translation, and manipulation were audited to ensure their accuracy. Data auditing involved 10% number-for-number checks against primary data sources initially, increasing to 100% number-for-number checks if significant errors were detected in the initial auditing step. Finally, statistical analyses of data were conducted to evaluate data distributions, identify appropriate summary statistics, and evaluate variability in the observations. Using these techniques, we were able to identify outliers and, if the outliers were due to error, correct the outlier values.

Our level of confidence about the shape and parameterization of the exposure distributions for total PCBc is directly related to sample size. The greater the number of whole body fish tissue samples, the more confidence in the exposure estimates generated from these data. Each of the AOCs had more than 17 fish sampled. Thus, our confidence in the exposure curves is high. All of the fish are carnivorous and consume similar prey items, though in different proportions.

Uncertainties in the Effects Assessment - The effects assessment is intended to describe the effects caused by stressors, link them to the assessment endpoints, and evaluate how effects change with fluctuations in the levels (i.e., concentrations or doses) of the various stressors. There are several sources of uncertainty in the assessment of effects including measurement errors, extrapolation errors, model fit errors, and data gaps.

The greatest source of uncertainty in the effects assessment for carnivorous fish is the few appropriate effects studies available where tissue residues were considered. Further, there were no studies that examined the effects of total PCBs on the focal species selected. Therefore, surrogate effects data on a mix of freshwater and marine fish species were used to compare to the body burdens in the focal species. It seems reasonable that the PCB thresholds for the focal species lie between the TRV bounds selected for this assessment. However, there is no way to verify this assumption without further studies being performed with the focal species specifically.

6.0 References

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Tables

Table F2-1. Chemicals of potential concern and area results from the conservative, deterministic risk assessment for carnivorous fish.

Chemicals of Potential Concern (COPCs)	Areas	Risk Quotient	Proceed to Probabilistic Risk Assessment?
Total Polychlorinated Biphenyls	Bayou d'Inde AOC	2.6	Yes
	Upper Calcasieu AOC	1.2	Yes
	Middle Calcasieu AOC	1	Yes
	Reference Areas	0.1	Yes[1]

[1] For comparison purposes only.

Table F2-2. Number of whole body fish samples and length ranges for each carnivorous fish focal species from each area.

Area of Concern (AOC)	Focal Species	Number of Samples	Length (mm)	
			Min	Max
Bayou d'Inde AOC	Red drum	14	319	440
	Black drum	1	309	309
	Spotted seatrout	19	>150	494
	Southern flounder	0	-	-
Upper Calcasieu River AOC	Red drum	5	350	385
	Black drum	7	235	385
	Spotted seatrout	13	230	475
	Southern flounder	0	-	-
Middle Calcasieu River AOC	Red drum	4	298	361
	Black drum	4	200	495
	Spotted seatrout	9	185	407
	Southern flounder	0	-	-
Reference Areas	Red drum	8	320	760
	Black drum	6	200	495
	Spotted seatrout	4	350	390
	Southern flounder	2	310	380

Min = minimum; Max = maximum.

Table F2-3. Whole body carnivorous fish wet weight and lipid normalized concentrations of total PCBs by area and species.

Area of Concern (AOC)	Focal Species	Tissue Concentration (mg/kg; Geo. Mean ± SD)	
		Wet Weight	Lipid Normalized
Bayou d'Inde AOC	Red drum	0.034 ± 0.059 (n=14)	6.97 ± 12.4 (n=14)
	Black drum	0.005 ± 0 (n=1)	0.5 ± 0 (n=1)
	Spotted seatrout	0.042 ± 0.117 (n=19)	6.067 ± 23.8 (n=18)
	Southern flounder	-	-
Upper Calcasieu River AOC	Red drum	0.014 ± 0.020 (n=5)	2.99 ± 4.021 (n=5)
	Black drum	0.053 ± 0.397 (n=7)	7.07 ± 15.1 (n=7)
	Spotted seatrout	0.037 ± 0.040 (n=13)	9.02 ± 23.6 (n=13)
	Southern flounder	-	-
Middle Calcasieu River AOC	Red drum	0.008 ± 0.012 (n=4)	1.31 ± 2.50 (n=4)
	Black drum	0.129 ± 0.324 (n=4)	26.0 ± 359 (n=4)
	Spotted seatrout	0.029 ± 0.047 (n=9)	6.0 ± 31.6 (n=9)
	Southern flounder	-	-
Reference Areas	Red drum	0.013 ± 0.018 (n=8)	12.790 ± 18.40 (n=8)
	Black drum	0.007 ± 0.014 (n=6)	4.118 ± 14.7 (n=6)
	Spotted seatrout	0.035 ± 0.025 (n=4)	23.6 ± 22.02 (n=4)
	Southern flounder	0.011 ± 0.015 (n=2)	11.4 ± 14.9 (n=2)

Geo. Mean ± SD = Geometric Mean plus/minus standard deviation; n = number of samples.
PCBs = polychlorinated biphenyls.

Table F2-4. Summary of studies examining the effects of fish species to PCBs.

Species	Life Stage	Exposure Route	Exposure Concen-tration (mg/kg)	Duration	Tissue Residue (mg/kg ww)	Effect Measured	% Effect	Comments	Reference	Note
Salmonidae										
Coho salmon	fingerling	diet	480	260 - 265	645 - 659	survival	100	fish died in the last 5 days of	Mayer *et al.* 1977	A1254
Oncorhynchus kisutch	fingerling	diet	48	265	54 - 57	growth	0	study no effect on growth detected	Mayer *et al.* 1977	A1254
Rainbow trout *Oncorhynchus mykiss*	juvenile	diet	100	330	81	survival / growth	0	no primary endpoint effects observed at any exposure concentration. Pigment changes and behavioral changes noted.	Nestel and Budd 1975	A1254
	adult	diet	200	60	5.13*	growth	29		Hendricks *et al.* 1981	A1254
	juvenile	diet	15	224	8.5	survival / growth	0	highest tissue residue taken from curve. No effects noted at any dietary concentration tested	Lieb *et al.* 1974	A1254
Brook trout	eyed-egg - fry	water	3.1 µg/L	127	125	survival	21	LOAEL mortality	Mauck *et al.* 1978	A1254
Salvelinus fontinalis	eyed-egg - fry	water	6.2 µg/L	127	284	survival	50	mortality observed 18 days post-hatch	Mauck *et al.* 1978	A1254
	eyed-egg - fry	water	13 µg/L	127	> 419	survival	100	mortality after 118 d. No tissue concentration reported in dead fish	Mauck *et al.* 1978	A1254
	eyed-egg - fry	water	1.5 µg/L	127	71	survival	0	NOAEL mortality	Mauck *et al.* 1978	A1254
	eyed-egg - fry	water	1.5 µg/L	127	<71	growth	0	growth reduced. Bone development	Mauck *et al.* 1978	A1254
	eyed-egg - fry	water	0.69 µg/L	127	31	growth	0	growth reduced at residue concentrations >31 µg/L	Mauck *et al.* 1978	A1254
	embryo	water	200 µg/L	21	77.9	hatch	22	hatch success was reduced 22% relative to controls	Freeman and Idler 1975	A1254

Table F2-4. Summary of studies examining the effects of fish species to PCBs.

Species	Life Stage	Exposure Route	Exposure Concen-tration (mg/kg)	Duration	Tissue Residue (mg/kg ww)	Effect Measured	% Effect	Comments	Reference	Note
Lake trout *Salvelinus namaycush*	fry	water (diet)	0.327 µg/L (22.6 µg/g)	176	26.3	growth	0		Berlin *et al.* 1981	A1254
	fry	water (diet)	0.021 µg/L (1.05 µg/g)	176	1.53	survival		survival reduced	Berlin *et al.* 1981	A1254
	fry	water (diet)	0.05 µg/L (0.72 µg/g)	52	4	survival / growth	100 / 0		Mac and Seeyle 1981	A1254
Cyprinidae										
Fathead minnow *Pimephales promelas*	<24 hr - adult	water	4.6 µg/L	240	741 - 1253	survival / growth	100 / 0		Nebeker *et al.* 1974	A1254
	<24 hr - adult	water	1.8 µg/L	240	83 - 553	reproduction			Nebeker *et al.* 1974	A1254
	<24 hr - adult	water	0.52 µg/L	240	54 - 133	reproduction	0		Nebeker *et al.* 1974	A1254
	6 months	water	71.3 µg/L	12.5	648 - 745	survival		survivial reduced / mortality	van Wezel *et al.* 1995	A1254
Ictaluridae										
Channel catfish *Ictalurus punctatus*	fingerling	diet	24	193	21	survival / growth	100 / 0	radiotracer study	Mayer *et al.* 1977	A1254
Cyprinodontidae										
Sheepshead minnow *Cyprinodon variegatus*	adult / egg	water	5.6 µg/L	28	170	survival	5		Hansen *et al.* 1973	A1254
	adult / egg	water	0.32 µg/L	28	5.1	survival	77		Hansen *et al.* 1973	A1254
	adult / egg	water	0.1µg/L	28	0.88	survival	97		Hansen *et al.* 1973	A1254

Table F2-4. Summary of studies examining the effects of fish species to PCBs.

Species	Life Stage	Exposure Route	Exposure Concen-tration (mg/kg)	Duration	Tissue Residue (mg/kg ww)	Effect Measured	% Effect	Comments	Reference	Note
Sparidae										
Pinfish	juvenile	water	5 µg/L	14	14	survival	66		Hansen *et al.* 1971	A1254
Lagodon rhomboides	juvenile	water	5 µg/L	35	109	survival	41		Hansen *et al.* 1971	A1254
	juvenile	water	100 µg/L	2	17	survival	0	embryos hatched and larvae raised in clean water	Hansen *et al.* 1971	A1254
Spot	juvenile	water	5 µg/L	20 - 26	46 - 120	survivial	51 - 53		Hansen *et al.* 1971	A1254
Leiostomus xanthurus	juvenile	water	5 µg/L	38	152	survival	62		Hansen *et al.* 1971	A1254
	juvenile	water	1 µg/L	33 - 56	17 - 27	survival	0		Hansen *et al.* 1971	A1254
Gasterosteidae										
3-spined stickleback *Gasterosteus aculeatus*	whole body	diet	NR - LD1	105	289	mortality	0		Holm *et al.* 1993	Clophen A50

* Hendricks *et al.* (1981) reported adult whole body burdens (Aroclor-1254) on a lipid normalized basis. No lipid content of the fish was reported.
Based on a literature search by Meador *et al.* (2002; Table 1) an average lipid content for adult whole body rainbow trout of 8.82% was used to convert the lipid normalized concentration to whole body wet weight concentration
ww = wet weight; PCBs = polychlorinated biphenyls.

Figures

Figure F2-1. Overview of approach used to assess exposure of carnivorous fish to contaminants of concern (COCs) in the Calcasieu Estuary.

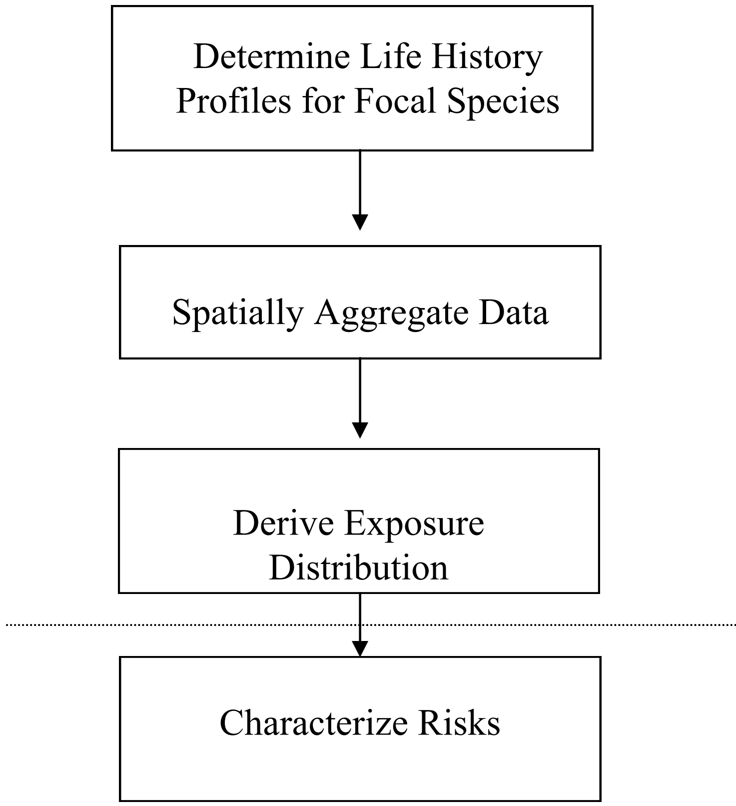

Figure F2-2. Overview of approach used to assess the effects of contaminants of concern (COCs) to carnivorous fish in the Calcasieu Estuary.

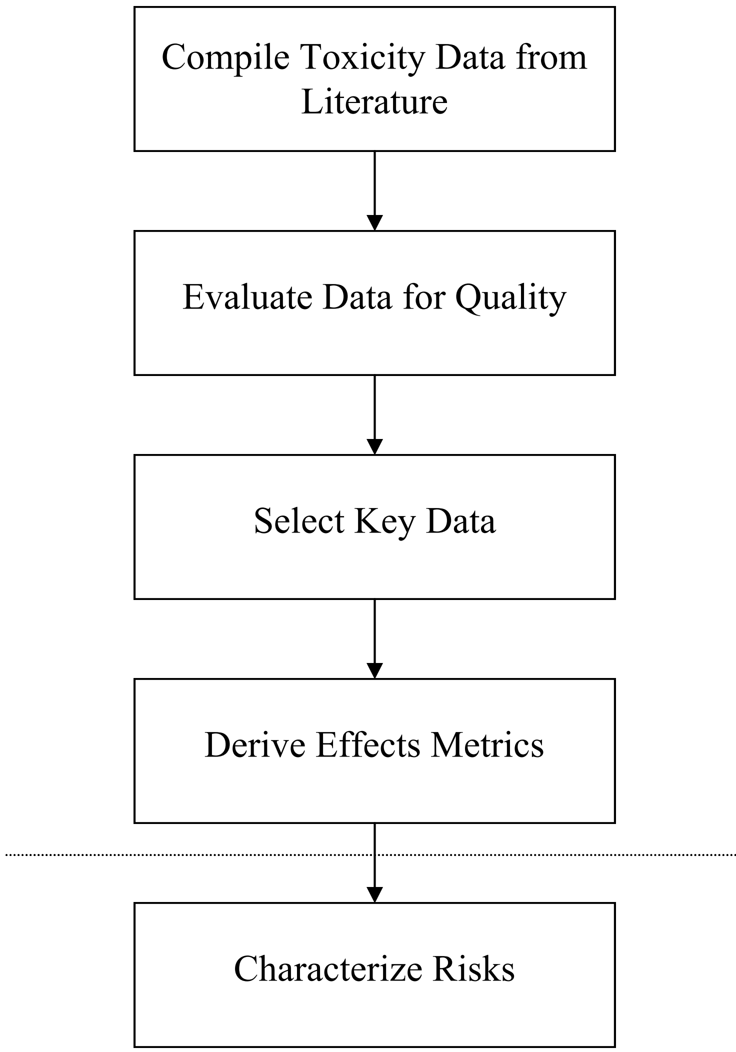

Figure F2-3. Overview of approach used to assess the risks of contaminants of concern (COCs) to carnivorous fish in the Calcasieu Estuary.

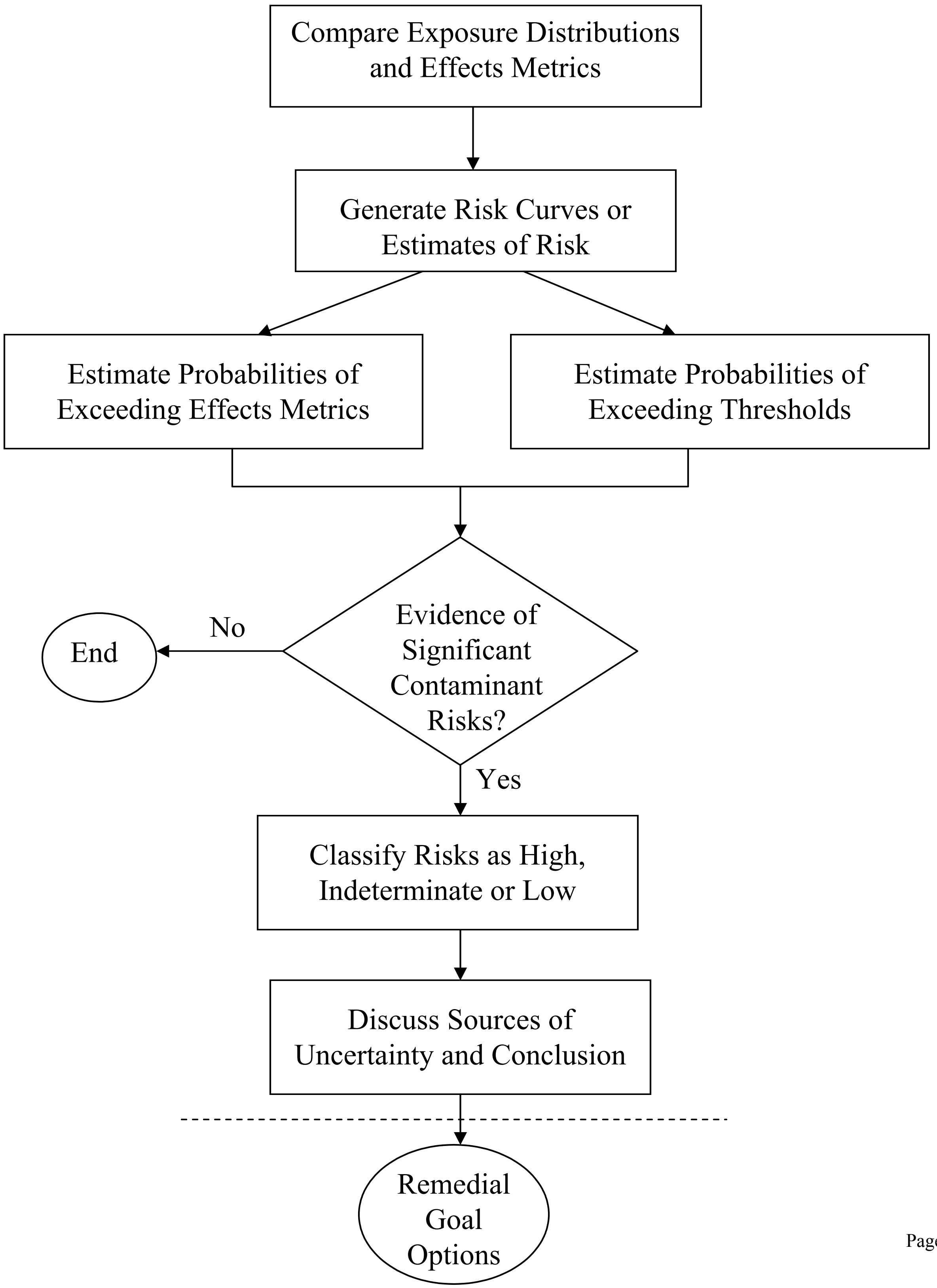

Figure F2-4. Annual geometric mean concentration of Aroclor 1254 in fish fillet from Upper Calcasieu River AOC, Calcasieu Estuary (bars represent minimum and maximum concentrations).

Figure F2-5. Annual geometric mean concentration of Aroclor 1254 in fish fillet from Bayou d'Inde AOC, Calcasieu Estuary (bars represent minimum and maximum concentrations).

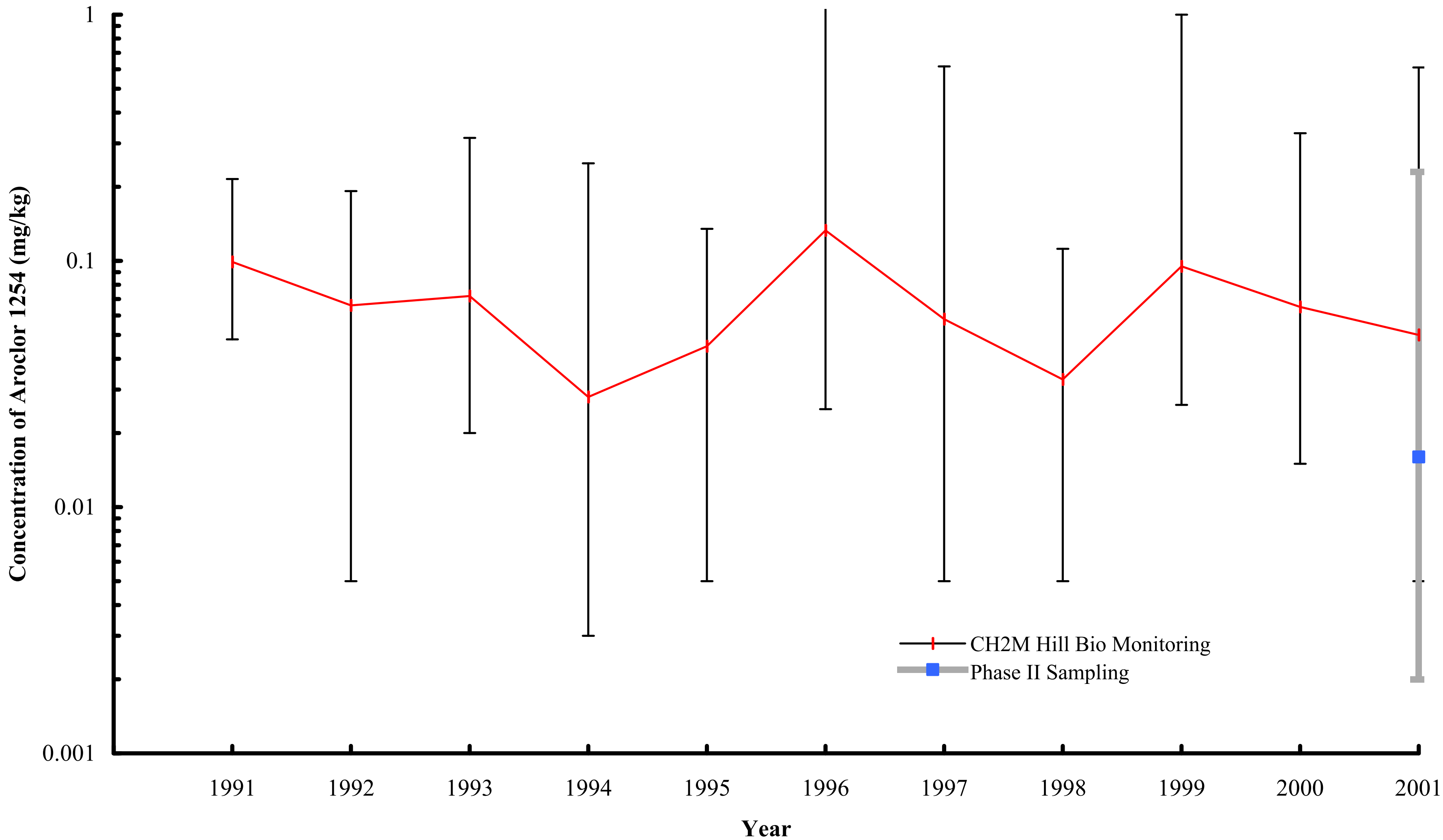

Figure F2-6. Annual geometric mean concentration of Aroclor 1254 in fish fillet from Middle Calcasieu River AOC, Calcasieu Estuary (bars represent minimum and maximum concentrations).

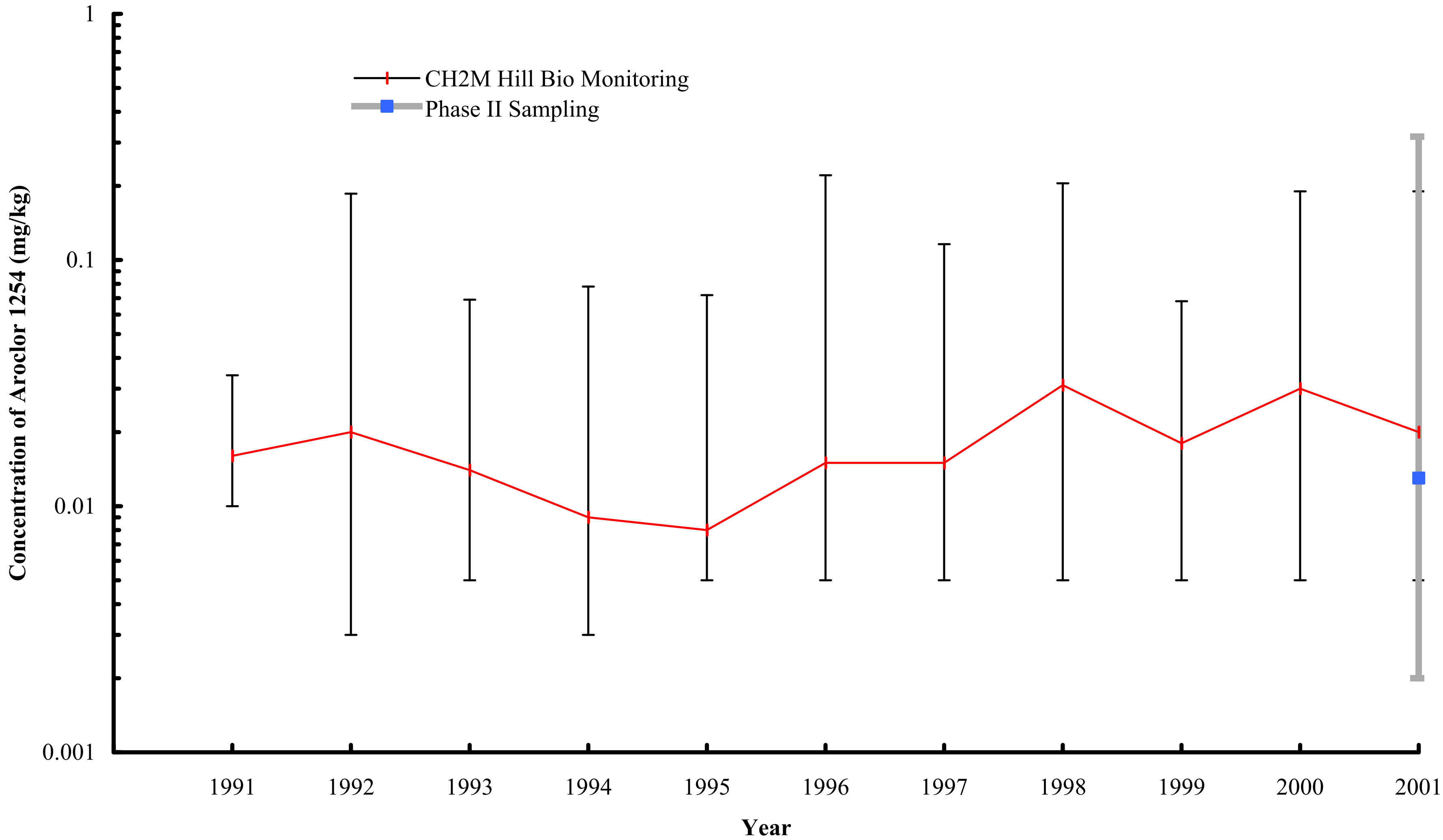

Figure F2-7. Annual geometric mean concentration of Aroclor 1254 in fish fillet from Reference Areas, Calcasieu Estuary (bars represent minimum and maximum concentrations).

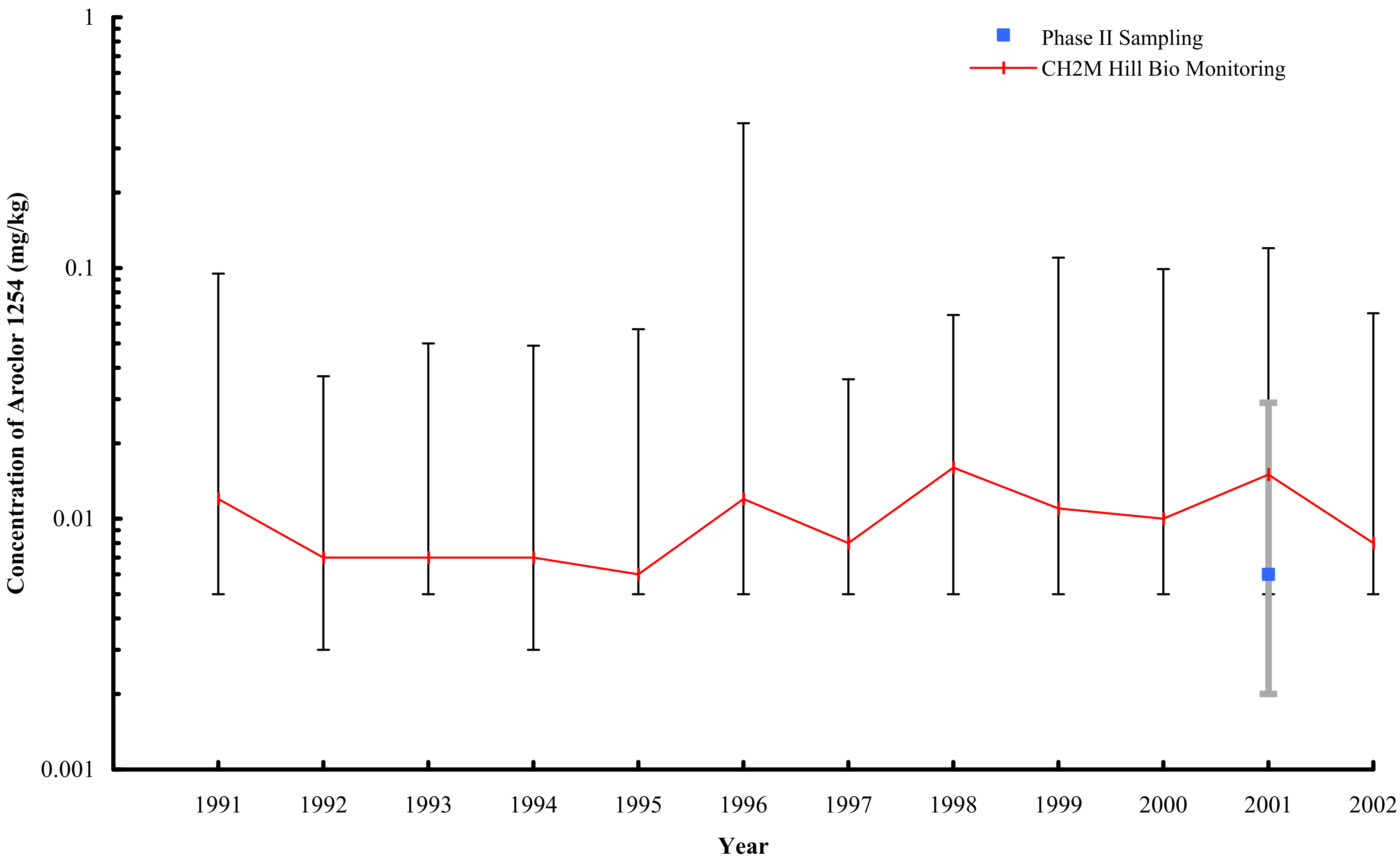

Figure F2-8. Summary of effects study results and comparison to the conservative, deterministic risk assessment screening benchmark.

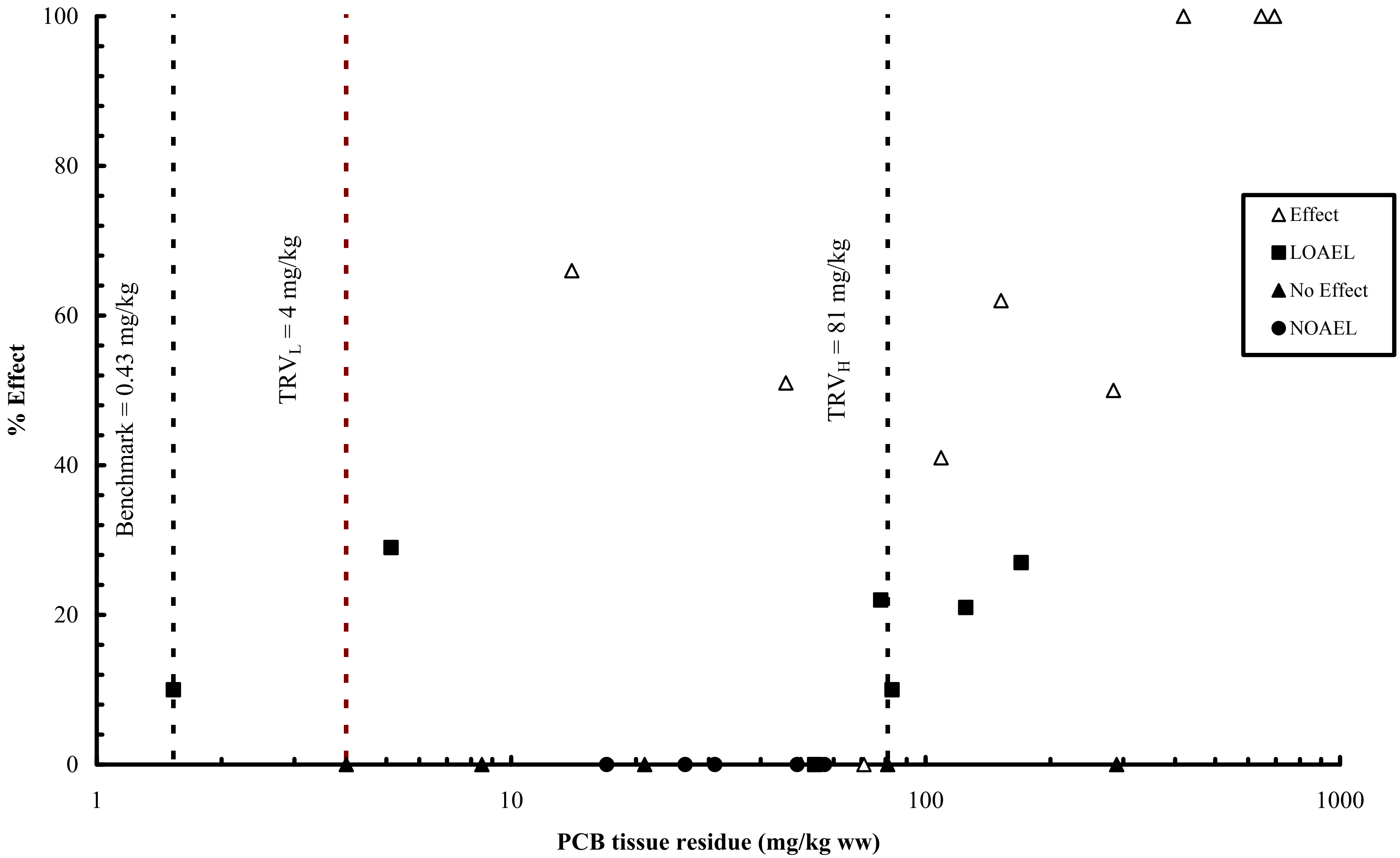

Figure F2-9. Total PCB body burden carnivorous fish in Bayou d'Inde AOC compared to low and high tissue thresholds (TRV_L and TRV_H).

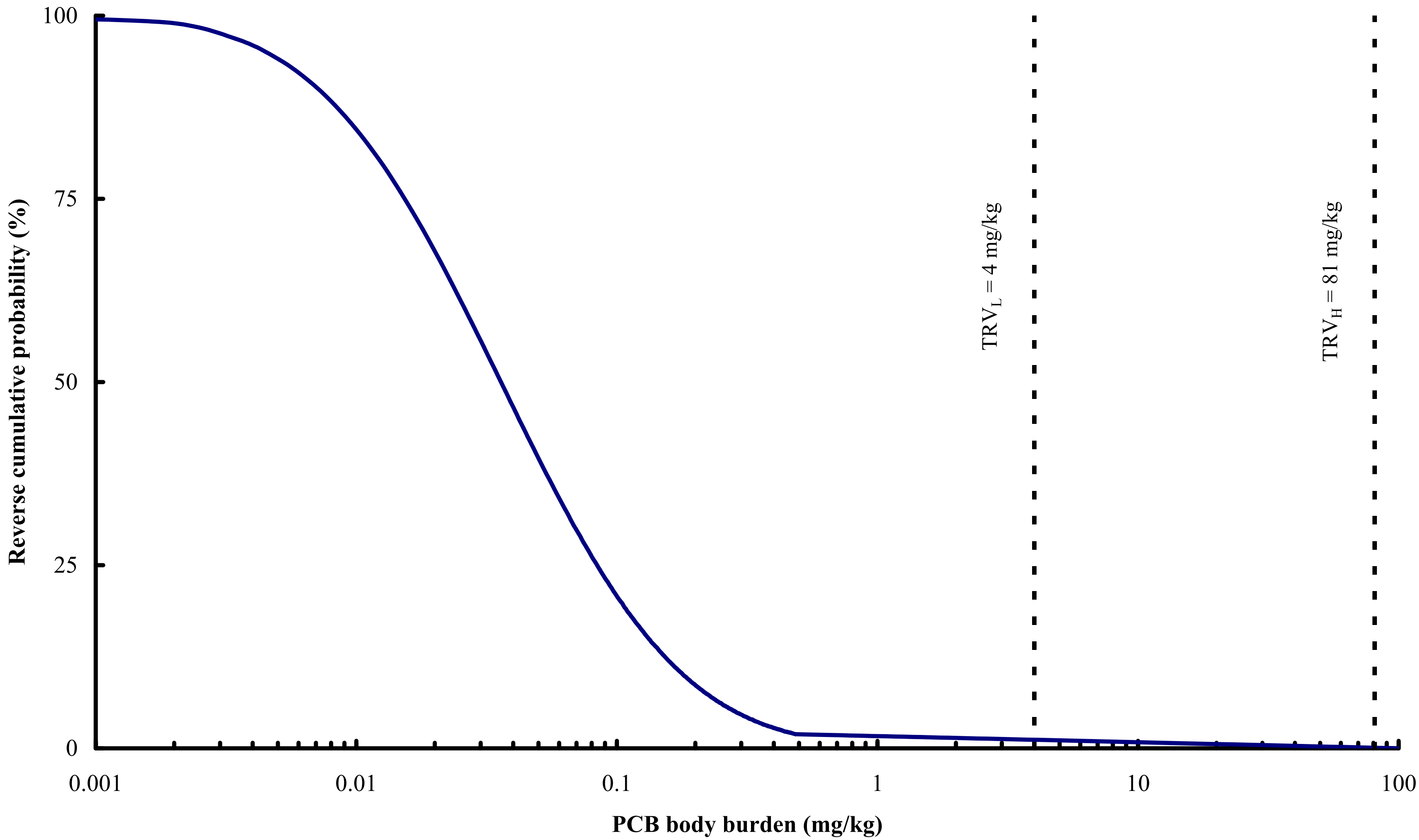

Figure F2-10. Total PCB body burden in carnivorous fish from the Reference Areas compared to low and high tissue thresholds (TRV_L and TRV_H).

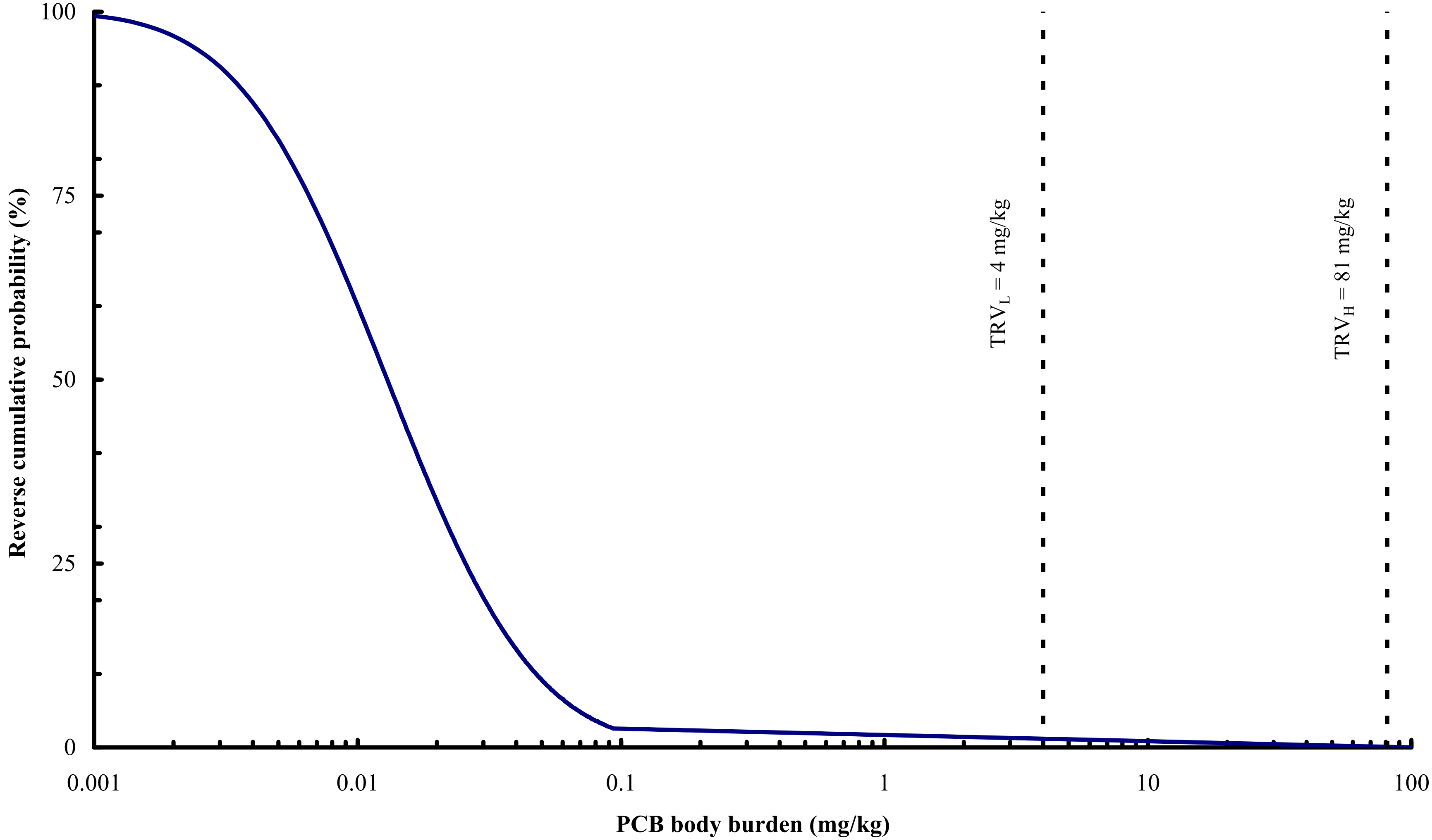

Figure F2-11. Total PCB body burden in carnivorous fish from the Upper Calcasieu AOC compared to low and high tissue thresholds (TRVL and TRVH).

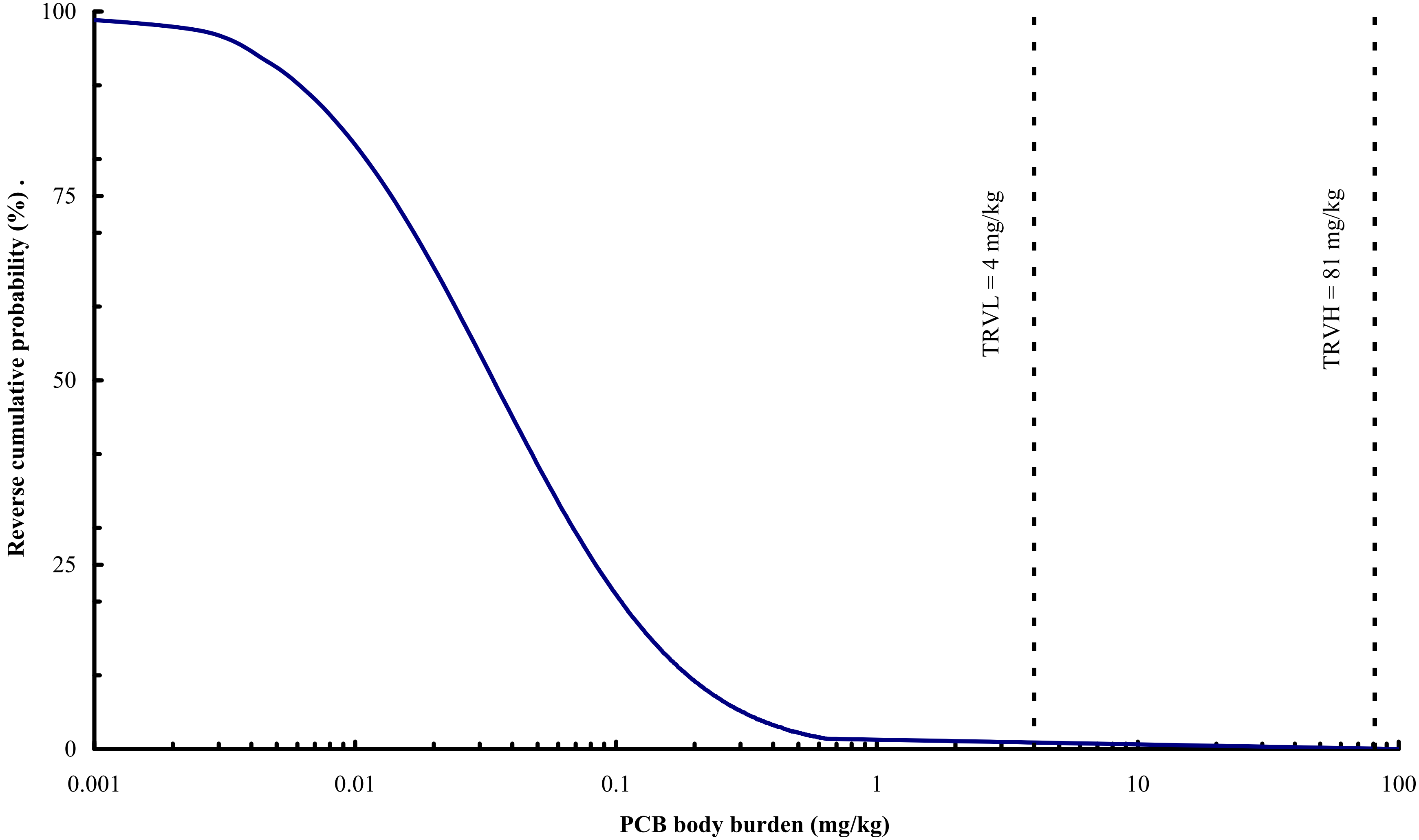

Figure F2-12. Total PCB body burden in carnivorous fish from the Middle Calcasieu AOC compared to low and high thresholds (TRV_L and TRV_H).